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# Influence of iodine supply on the radiation-induced DNA-fragmentation

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ABSTRACT

The protective effect of stable iodide against radiation on thyroid cells was investigated. One physiological effect of stable iodine is well-rooted: stable iodine leads to a reduced thyroid uptake of radioactive iodine. This work wants to focus on an intrinsic effect of stable iodine by which DNA-damage in cells is prevented.

To investigate this intrinsic effect thyroid cells (FRTL-5) were externally irradiated by use of a linear accelerator (LINAC) applying energy doses of 0.01 Gy–400 Gy and by incubation with various activity concentrations of $^{131}$I (0.1–50 MBq/ml for 24 h). We added stable iodine (NaI) to the cells prior to external irradiation and investigated the effect of the concentration of stable iodine (1, 5, 15 μg/ml). In order to clarify whether thyroid cells have a distinctive and iodine-dependent reaction to ionizing radiation, keratinocytes (HaCaT) without NIS were exposed in the same way. As indicators for the cellular reaction, the extent of DNA fragmentation was determined (Roche, Mannheim, Germany).

Both cell types showed distinct ability for apoptosis as proven with camptothecin. The addition of "cold" iodine from 1 to 15 μg/ml without irradiation ("negative control") did not change the response in both cell types. Plausibly, the radio-sensitivity of both cell types did increase markedly with increasing radiation dose but the radiation effect is diminished if iodine is added to the thyroid cells beforehand. The DNA-damage in thyroid cells after addition of cold iodine is reduced by a factor of 2–3. The skin cells did not show an significant change of radio-sensitivity depending on the presence of cold iodine. Elementary iodine possibly acts as a radical scavenger and thus markedly reduces the secondary radiation damage caused by the formation of cytotoxic radicals. This intrinsic radioprotective effect of iodine is seen only in cells with NIS.



## 1. Introduction

Non-radioactive iodine and thyrostatic drugs have long been known to have a significant influence on the therapeutic outcome of radioidine therapies [Schomäcker et al., 1996] and it is common knowledge that stable iodine ("cold iodine") competes with radioactive isotopes of iodine ("hot iodine"). The uptake of radioiodine may be significantly reduced when the concentration of stable iodine in the thyroid is high [Ramsden et al., 1967]. But apart form this well-know fact ("competiton effect") it remains unclear if the competition of cold and hot iodine is the only influence of cold iodine on the radiation sensitivity of cells. The aim of this work is to demonstrate that the iodine supply has an additional effect on the radiosensitivity of thyroid cells as cold iodine furthermore prevents DNA damage. We therefore studied the DNA fragmentation in irradiated cells after addition of iodine in physiological concentrations (μmol/l).

The uptake of iodine into thyroid cells is regulated by the NaI-symporter (NIS) and depends on the iodine concentration [Kogai and Brent, 2012]. The radioiodine uptake in cells is limited by the presence of stable iodine due to competition for the NIS ("competition effect") and inhibition of NIS expression, transient block of organification, and inhibition of hormonal release. This limitation of radioiodine uptake is undesirable for radioiodine therapy [Min et al., 2001], as it reduces the energy dose deposited in the thyroid and hence lessens the therapeutic effect. Conversely, it has

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been demonstrated that sufficient iodine supply reduces the risk of radiation-induced thyroid cancer after accidental exposure to radioiodine [Shakhtarin et al., 2003, Gembicki et al., 1997, Reiners and Schneider, 2013, Schneider and Smith, 2012]. This could also be explained by the reduced uptake of radioiodine due to the competiton effect. It is unclear whether iodine has an influence on its own to the radiosensitivity of cells due to its chemical properties, e.g. as a radical scavenger [Zhang et al., 2006, Yao et al., 2012].

Ionizing radiation transfers energy to cells either directly via cellular components and molecules or indirectly via water-derived radicals and reactive oxygen species (ROS) produced by radiolysis of water molecules. The latter react with nearby molecules within microseconds, resulting in breakage of chemical bonds or oxidation. A decisive contribution to radiation-induced cell damage is made by DNA breaks. Since DNA consists of a pair of complementary double strands cleavage can occur in either one single strand or both. However, the latter are believed to be much more critical biologically. Dose-effect relationships have been well established for several decades, with various indicators being used to quantify the effect induced by the radiation [Streffer and Müller 1987].

The use of CDDT for evaluation of radiation-induced cell damage is based on the detection of DNA-fragments as an indicator for cellular reactions. In recent years this test has become a commonly used measure of radiation-induced DNA fragmentation [Fischer et al., 2012]. The method was originally developed for detection of apoptosis. The splitting of DNA into oligonucleotides is taken as an indicator of the apoptotic process performed by specific endonucleases at the end of the signal cascade. Internucleosomal sections of DNA are split into histone-bound mono- or oligonucleosomes with about 180 base pairs, which can then be detected in the cytoplasm by means of anti-histone and anti-DNA antibodies. By the CDDT it is not feasible to distinguish whether the DNA strand breaks are directly generated by radiation or is a consequence of apoptotic pathways initiated by irradiation.

Thyroid cells were externally irradiated by use of a linear accelerator (LINAC) and after incubation with various concentrations of radioiodine ($Na^{131}I$). For the different types of radiation exposure we investigated the influence of the concentration of stable iodine added to the cells prior to irradiation. In order to clarify that thyroid cells have a distinctive reaction to ionizing radiation, keratinocytes without NIS were exposed in the same way. The extent of DNA damage was determined as an indicator of the cellular reaction.

## 2. Materials and methods

### 2.1. Preparation of cells

Thyroid cells of the Fischer rat thyroid cell line FRTL-5 show the typical behaviour of thyroid cells (TSH-dependent growth, NIS expression) and are therefore suitable for studying the iodine dependence of radiation sensitivity. FRTL-5 (provided by B. Meller, Halle and Göttingen, Germany) and human adult low calcium temperature keratinocytes (HaCaT) purchased from the German Cancer Research Centre (DKFZ, Heidelberg, Germany) were cultivated and prepared for irradiation in the way described by Fischer et al., 2012].

The number of cells was counted using a Fuchs-Rosenthal counting chamber (LO Laboroptik, Friedrichstal, Germany). $3^{*}10^{5}$ cells of both cell types were immersed in 10 ml culture medium. 100 l of this suspension was transferred into each well of a 96-well microtiter plate (Corning BV Life sciences, Amsterdam, The Netherlands). These plates were placed in an incubator under the conditions described (24 h). After a further 24 h 100 µl of medium was added. Each transfer was carried out in an isolated box.

A test of the CDDT was carried out with an alkaloid (camptothecin, CPT) which is known to induce apoptosis.

### 2.2. Irradiations

External beams irradiations were performed with linear accelerators from a radiation therapy department of the University Hospital of Cologne. For organisational reasons two different LINACs were used for cell irradiations (Elekta SL 75-5, Elekta SLi). The Elekta SL 75-5 produces 5 MeV photon beams and the Elekta SLi (Elekta, Innsbruck, Austria) produces 6 MeV photon beams.

The field dimensions were $38 \times 38$ or $36 \times 36$ $cm^2$, respectively. The source-surface distance was 58.5 or 57.2 cm, respectively. 10 cm of polystyrene was used as back-scatter material. Isocentric irradiations were performed from different angles. Irradiations were performed using energy doses of 0.01, 0.11, 0.12, 1.0, 4.4, 5, 9.6, 12.5, 21, 25, 45.8, 50, 96, 100, 200 and 400 Gy.

For irradiation with radioiodine in the chemical form of $Na^{131}I$, the cells were incubated with four different radioactivity concentrations of $^{131}I$ (0.1, 1, 10 and 50 MBq/ml $^{131}I$) for 24 h.

### 2.3. Detection of induced cellular reactions

Nucleosomal DNA was assayed in the culture supernatant and in the cytoplasmic fraction by an enzyme-linked immunosorbent assay employing the Cell Death Detection ELISAplus kit (Cell death detection test CDDT, Roche, Mannheim, Germany) according to the manufacturer's instructions. The photometric absorption (extinction) of samples and negative controls was determined using a SPECTRA classic reader (SLT, Salzburg, Austria) at 405 nm (reference wavelength 492 nm). The results are discussed as "enrichment factor". This factor describes the ratio of absorption of irradiated cells and non-irradiated cells.

The fundamental ability of both cell types to undergo apoptosis and the sensitivity of CDDT in detecting this were tested systematically by means of the Camptothecin-test (CPT-test). CPT is an alkaloid that causes single and double strand breaks in DNA by preventing the re-ligation of DNA and inducing apoptosis. CPT was added in the following concentrations: 0.1, 1, 2 and 4 mg/ml medium.

### 2.4. The intrinsic influence of "cold" iodine

For each experiment (LINAC and $^{131}I$) a control group was irradiated after addition of "cold" anionic iodine form in the respective concentrations and incubated in the same way. The difference in absorption of irradiated and non-irradiated cells was calculated as described.

### 2.5. Dosimetry

Different approaches were applied for the dose calculations for external and internal irradiations. In the case of incubation with $Na^{131}I$ dosimetry was based on the Monte Carlo N-Particle code (MCNP) (http://mcnp-green.lanl.gov/index.html) in the form of the EGS-ray code [Kleinschmidt, 2001]. Simulations were performed assuming an exposure time of 24 h and a cylindrical well geometry with a volume of 200 µl (height: 0.57 cm, radius: 0.335 cm). The cells were assumed to build a layer on the base of the cylinder (height: 0.001 cm, radius: 0.3 cm). Calculations were performed for the beta particles from I-131 in 10 keV intervals with a range from 0 to 810 keV. The release of γ-rays was ignored because the specific absorbed fraction is low and γ-rays therefore do not contribute significantly to the dose. $10^{6}$ single decays emitting electrons according to the β-spectrum of $^{131}I$ yielded individual tracks in the

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